WHAT IS CLAIMED IS:

1. A polynucleotide which, upon introduction into a mammalian cell induces the co-expression in the cell of at least two gene products, comprising:

a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron;

a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;

optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and

a transcriptional terminator following each of the first, second and third cistron.

2. The polynucleotide of Claim 1 wherein the first cistron encodes at least one immunogenic epitope of a pathogen or a cancer associated antigen.

3. The polynucleotide of Claim 2 wherein the pathogen is a virus.

4. The polynucleotide of Claim 3 wherein the virus is the human immunodeficiency virus (HIV).

5. The polynucleotide of Claim 2 wherein the first cistron encodes a human immunodeficiency virus (HIV) gene selected from env, gag, gag/pol, gag/protease, gag and portions of pol not encoding a functional polymerase, and pol.

6. The polynucleotide of Claim 1 wherein the second cistron encodes a human immunodeficiency virus (HIV) REV gene if the first cistron encodes an HIV gene, the efficient expression of which is dependent on availability within the cell expressing the HIV gene of the REV gene product.

7. The polynucleotide of Claim 6 wherein the first cistron encodes an HIV late gene selected from env, gag and pol.

8. The polynucleotide of Claim 7 wherein the first cistron encodes HIV gp160, HIV gp120, HIV gp41, HIV gp120 lacking a CD4 binding site and HIV env with an immunologically altered V3, the altered V3 having an altered glycosylation pattern or substituted V3 loop tips.

9. The polynucleotide of Claim 6 wherein the third cistron encodes a cytokine or a T-cell costimulatory element.

10. The polynucleotide of Claim 9 wherein the cytokine is interferon, GM-CSF, or interleukin.

11. The polynucleotide of Claim 9 wherein the T-cell costimulatory element is a gene encoding a B7 protein.

12. The polynucleotide of Claim 1 wherein the first cistron encodes a REV-independent human immunodeficiency (HIV) epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein each of the cistrons may also be presented in a different order.

13. The polynucleotide of Claim 12 wherein the second cistron encodes an interleukin, an interferon, or GM-CSF, and the third cistron encodes a B7 protein.

14. The polynucleotide of Claim 1 wherein either of the second and third cistron is under transcriptional control of the transcriptional promoter upstream of the first cistron, a sequence is provided upstream of each of the second and third cistrons having the function of an internal ribosome entry site (IRES) to effect efficient translation of the second and third cistrons on a bi- or tri-cistronic messenger RNA transcribed from the beginning of the first cistron through each of the second and third cistrons up to the transcriptional terminator following the second or third cistron.

15. The polynucleotide of Claim 14 wherein the IRES is selected from encephalomyocarditis virus (EMCV) IRES, swine vesicular virus IRES and poliovirus IRES.

16. The polynucleotide of Claim 14 wherein the first cistron encodes a human immunodeficiency virus (HIV) REV dependent gene, the second cistron encodes REV, and the third cistron encodes a T-cell costimulatory element or a cytokine, and further, wherein the first cistron is preceded by a transcriptional promoter and the second and third cistrons are each preceded by an IRES and no transcriptional promoter.

17. The polynucleotide of Claim 16 wherein the first cistron encodes an HIV gp160, the first cistron is preceded by cytomegalovirus immediate early promoter, the second cistron encodes HIV REV, the optional third cistron encodes an interferon, GM-CSF, an interleukin, or a B7 protein.

18. A polynucleotide which comprises contiguous nucleic acid sequences which cannot replicate in eukaryotic cells but which are capable of being expressed to produce a gene product upon introduction of the polynucleotide into eukaryotic tissues <u>in vivo</u>, wherein the gene product either acts as an immunostimulant or as an antigen capable of generating an immune response, wherein the nucleic acid sequences encode:

a spliced REV gene;

a spliced human immunodeficiency virus (HIV) immunogenic epitope; and

optionally, a cytokine or a T-cell recognition element.

19. The polynucleotide of Claim 18 wherein the HIV immunogenic epitope selected from gag, gag-protease, or env or an immunogenic subportion thereof; the cytokine is interleukin-12, and the T-cell costimulatory element is a B7 protein

20. The polynucleotide of Claim 19 wherein the env immunogenic epitope is selected from HIV gp160, HIV gp120 and HIV gp41.

21. The polynucleotide of Claim 19 wherein the gag immunogenic epitope is p17, p24, or p15.

22. A polynucleotide comprising a first gene encoding an HIV gag, gag-protease, or env immunogenic epitope, the gene containing a REV responsive element (RRE) or having been modified to contain an RRE, the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a gene encoding a REV gene product.

23. The polynucleotide construct:

a) **V1Ins-*rev*IIIB**, which has the junction sequence SEQ.ID:56:

5'-GGA GAC AGC GACGAA GAC CTC CTC AAG GCA GTC AGA CTC ATC AAG-3', and SEQ.ID:57:

5'-GAT GGC TGG CAA CTA GAA GGC ACA GCA GAT CT/ GAT ATC GCA CTA

BGH ***rev...***

TTC TTT AGC TCC TGA CTC CAA TAT TGT-3'

b) **V1Jns-gp160IIIB**, which has the junction sequence SEQ.ID:58:

5'-CTT AGA TC/ A ACC ATG AGA GTG AAG GA GAA ATA TCA GCA CTT GTG
CMVinta **gp160**

GAG ATG GGG GTG GAG ATG GGG CAC CAT GCT CCT TGG GAT GTT GAT GAT CTG
TAG TGC TAC AGA AAA ATT GTG GGT-3',

and SEQ.ID:59:
5'-CTG GCA ACT AGA AGG CAC AGC AGA TC/ A GAT AGT GTC CCC ATC TTA
BGH **gp160**

TAG CAA AAT CCT TTC CAA GCC CTG TCT TAT TCT-3'

c) **pGEM-3-IRES**, which has the junction sequence SEQ.ID:62:

5'-CAT GCC TGC AGG TCG ACT CTA/ AAT TCC G...
pGEM-3 (SP6) **IRES**

and SEQ.ID:63:
5'-A CCC GGG GAT CCT CT/ A GCG CGC TTG TCT CTT GTT CCA...
pGEM-3 (T7) **IRES**

d) **pGEM-3-IRES/*rev*IIIB**, which has the junction sequence SEQ.ID:65:

5'-TAT GGC CAC AAC C/ AT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA
IRES ***rev***
CCT CCT CAA GGC AGT CAG ACT -3'

and SEQ.ID:66:
5'-CTC GAG CCA TGG GCC CCT/ AGA CTA TAG CGT GAT AAG AAA TCG AGG
pGEM-3 ***rev***

ACT GAG GTT ATA ACA TCC TCT AAG GTG GTT ATA AAC TCC CGA AGG-3'

e) **pGEM-3-RRE/IRES/*rev*IIIB**, which has the junction sequence SEQ.ID:68:

5'-TTG CAT GCC TGC AGG T/ GGT ACA TGA TCA GAT ATC G CCC GGG / C

pGEM-3 **RRE**

CGA GAT CTT CAG ACT TGG AGG AGG AGA TAT GAG GGA CAA TTG GAG-3'

IRES-5'

and SEQ.ID:69:

5'-GGG GCG GAA TT/ T AGA GTC A/ ATT GAT CAG CTT GTG TAA TTG TTA

RRE-3'

ATT TCT CTG TCC CAC TCC ATC CAG GTC GTG TGA TTC...-3'

f) **V1Jns-(*tat/rev* SD)**, which has the junction sequence SEQ.ID:71:

5'-AGA TCT A AGG ACG GTG ACT GCA / TGT ACT ACT TAC TGC TTT GAT

CMVintA ***tat/rev* SD**

AGA GGA CGG TGA / CTG CAG AAA AGA CCC ATG GAA A-3'

CMVintA

g) **V1Jns-gp160IIIB/IRES/*rev*IIIB (SD)**, which has the junction sequence SEQ.ID:73:

5'-GGC ACA GCA GAT C/ AG ATG GGG ATC TGA TA TCG CAC TAT TCT TTA

BGH ***rev***

GCT CCT GAC TCC TGA CTC-3'

and SEQ.ID:74:

5'-GGA ATT/ TGA GTC ATC / CCC ATC TTA TAG CAA AAT CCT TTC CAA -3'

IRES **gp160**

h) **V1Jns-*gag-prt*IIIB (SD)**, which has the junction sequence SEQ.ID:75:

5'-CTT AGA TC/ C CCG CAC GGC AAG AGG CGA GGG GCG GCG ACT GGT-3'
CMVintA ***gag*** **(SD)**

and SEQ.ID:76:
5'-GGC ACA GCA GAT C/ CGC CCG GGC TTA CAT CTC TGT ACA AAT TTC TAC
BGH ***prt***

TAA TGC TTT TAT TTT TCT TCT GTC...-3'

i) **V1Jns-*gag*-*prt*IIIB**, which has the junction sequence SEQ.ID:77:

5'-CTT AGA TC/ CAC CAT GGG TGC GAG AGC GTC AGT ATT AA GCG GGG
CMVintA ***gag***

GGA GAA TTA GAT CGA TGG GAA AAA ATT...-3'

and SEQ.ID:78:
5'-GGC ACA GCA GAT C/ CGC CCG GGC TTA CAT CTC TGT ACA AAT TTC TAC
BGH ***prt***

TAA TGC TTT TAT TTT TCT TCT GTC...-3'

j) **V1Jns-tPA**, which has the junction sequence SEQ.ID:79:
5'-TCA CCG TCC TTA GAT C/ ACC ATG GAT GCA ATG AAG AGA GGG CTC TGC
CMVintA **tPA leader**

TGT GTG CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GA/ G ATC
BGH

TGC TGT GCC TTC TAG TTG CCA GCC-3'

k) **V1Jns-tPA-gp120MN**, which has the junction sequence SEQ.ID:80:

5'-TTC GTT TCG CCC AGC GA/ TCA CAG AAA AAT TGT GGG TCA CAG TC-3'
tPA **gp120MN**

and SEQ.ID:81:

5'-GGC ACA GCA GAT C/ CAC GTG TTA GCG CTT TTC TCT CTC CAC CAC-3'

BGH **gp120MN**

l) **V1J-SIVMAC251 p28 *gag*** , which has the junction sequence SEQ.ID:82:

5'-TCA CCG TCC TTA GAT CT/ ACC ATG GGA CCA GTA CAA CAA ATA GGT

CMVintA **p28 *gag*...**

GGT AAC TAT GTC CAC CTG CCA TTA AGC CCG AGA ACA-3'

and SEQ.ID:83:

5'-GGC ACA GCA GAT CT/ TTA CAT TAA TCT AGC CTT CTG TCC CGG TCC-3'

BGH **p28 *gag***

m) **V1J-SIVMAC251*nef***, which has the junction sequence SEQ.ID:84:

5'-TCA CCG TCC TTA GAT C/ GGT ACA ACC ATG GGT GGA GCT ATT TCC ATG

CMVintA ***nef*.....**

AGG CAA TCC AAG CCG GCT GGA GAT CTG ACA GAA A-3'

and SEQ.ID:85:

5'-GGC ACA GCA GAT CA/ C CTA GGT TAG CCT TCT TCT AAC CTC TTC CTC

BGH ***nef*....**

TGA CAG GCC TGA CTT GCT TCC AAC TCT TCT GGG TAT CTA G-3'

n) **V1Jns-*tat/rev/env***: , which has the junction sequence SEQ.ID:86:

5'-ACC GTC CTT AGA T/ TC GAC ATA GCA GAA TAG GCG TTA CTC GAC AGA

CMVintA ***tat/rev/env***

GGA GAG CAA GAA ATG GAG CCA GTA GAT CCT AGA CTA GAG CCC TGG-3'

and SEQ.ID:87:

5'-GGC ACA GCA GAT C/ C GAG ATG CTG CTC CCA CCC CAT CTG CTG-3'.

BGH ***tat/rev/env***

24. A polynucleotide which induces anti-HIV neutralizing antibody, HIV specific T-cell immune responses, or protective immune responses upon introduction into vertebrate tissue, including human tissue in vivo, wherein the polynucleotide comprises a gene encoding a gene product selected from HIV gag, HIV gag-protease, and HIV env, the gene containing a REV responsive element (RRE), the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene, the second gene encoding a REV gene product.

25. A method for co-expression in a single cell in vivo, of at least two gene products, which comprises introducing between about 1 ng and about 100 mg of the polynucleotide of Claim 1 into the tissue of the vertebrate.

26. A method for inducing immune responses in a vertebrate against HIV epitopes which comprises introducing between about 1 ng and about 100 mg of the polynucleotide of Claim 6 into the tissue of the vertebrate.

27. A method for inducing immune responses in a vertebrate against HIV epitopes which comprises introducing between about 1 ng and about 100 mg of the polynucleotide of Claim 14 into the tissue of the vertebrate.

28. A method for using a REV dependent HIV gene to induce immune responses in vivo which comprises:

a) isolating the REV dependent HIV gene;

b) linking the isolated gene to regulatory sequences such that the gene is expressible by virtue of being operatively linked to control sequences which, when introduced into a living tissue, direct the transcription initiation and subsequent translation of the gene;

c) introducing the expressible gene into a living tissue; and

d) introducing a gene encoding HIV REV either in trans or in cis to the HIV REV dependent gene.

29. The method of Claim 28 which further comprises boosting with additional expressible HIV gene, or boosting with a recombinant purified HIV gene product.

30. The method of Claim 28 wherein the REV-dependent HIV gene encodes a gag, or an env gene product.

31. A method for inducing immune responses against infection or disease caused by virulent strains of HIV which comprises introducing into the tissue of a vertebrate an HIV gene from a first HIV strain such that an induced immune response neutralizes infection by the first HIV strain but also neutralizes infection by strains heterologous to the first strain, wherein the HIV gene encodes a conserved, REV dependent HIV epitope and a functional REV is provided either in cis or in trans.

32. A vaccine for inducing immune responses against HIV infection which comprises the polynucleotide of Claim 1 and a pharmaceutically acceptable carrier.

33. A method for inducing anti-HIV immune responses in a primate which comprises introducing the polynucleotide of Claim 1 into the tissue of the primate and concurrently administering interleukin 12 parenterally.

34. The method of Claim 33 wherein the first cistron of the polynucleotide encodes HIV gp160, the second cistron of the polynucleotide encodes HIV REV, and the third cistron of the polynucleotide encodes B7.

35. A polynucleotide comprising:

a) an eukaryotic transcriptional promoter;

b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open reading frame, and a stop codon encoding the termination of translation of the open reading frame;

c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;

d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;

e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes, the genes being selected from GM-CSF, IL-12, interferon, and a B7 protein;

f) a transcription-termination signal following the last open reading frames.

36. A method of inducing an antigen-presenting cell to stimulate cytotoxic and helper T-cell proliferation effector functions, the functions comprising lymphokine secretion specific to HIV antigens, the method comprising:

a) exposing cells of a vertebrate in vivo to a polynucleotide, the polynucleotide comprising sequences encoding an antigenic HIV epitope, optionally, HIV REV, and sequences encoding a B7 protein.

37. The method of Claim 36 wherein the HIV epitope is selected from env, gag, and pol.

38. The method of Claim 36 wherein the polynucleotide encodes an IRES between each of the HIV epitope, the REV, and the B7 protein.

39. A polynucleotide which comprises sequences encoding:

a) an eukaryotic transcription initiation signal;

b) an HIV gene open reading frame (ORF) preceded by an heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;

c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;

d) a sequence encoding an ORF of a T-cell costimulatory element 3' to the IRES; and

e) a transcription termination signal 3' to the translation stop codon of the T-cell costimulatory element.

40. The polynucleotide of Claim 39 wherein the HIV gene ORF in (b) is tPAgp120 or tPAgp160.

41. A polynucleotide which comprises sequences encoding:

a) an eukaryotic transcription initiation signal;

b) an HIV gene open reading frame (ORF) preceded by an heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;

c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;

d) an HIV gene open reading frame (ORF) preceded by an heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product; and

e) a transcription termination signal 3' to the translation stop codon of the HIV gene ORF.

42. A composition comprising multiple expression constructs each of which is capable of inducing expression in mammalian tissue of more than a single cistron encoding antigens related to disease causing pathogens or tumors.

43. A method for immunization of a host vertebrate comprising the step of:
introducing into direct contact with tissue of the host a non-infectious, non-integrating polynucleotide encoding at least a first and a second peptide or polypeptide, each of which is immunogenic or immunomodulatory when produced as a translation products in the host wherein the first peptide or polypeptide is encoded by a segment of the polynucleotide which is under operative control of a first transcriptional promoter and the second peptide or polypeptide is encoded by a segment of the polynucleotide under operative control of the first transcriptional promoter, in which case no transcriptional terminator is provided between the polynucleotide segment encoding the first peptide or polypeptide and the segment of polynucleotide encoding the second peptide or polypeptide, or the second peptide or polypeptide is encoded by a segment of the polynucleotide under operative control of a second transcriptional promoter, in which case a transcriptional terminator is provided between the segment of polynucleotide encoding the first peptide or polypeptide and the segment of polynucleotide encoding the second peptide or polypeptide, whereby both of the first and second peptide or polypeptide are produced within a single cell of the host, resulting in the immunization.

44. A polynucleotide construct having the elements shown in figure 2, wherein each of the first, second and third cistrons shown in the figure encode a combination of any two to three of the following:

1) tPA-gp120MN;
2) gp160IIIB/IRES/*REV*IIIB;
3) gp160IIIB;
4) *REV*IIIB;
5) *tat*/*REV*/gp160;
6) REV/gp160;
7) gp160MN;
8) gp160 from clinically relevant primary HIV isolates;
9) nef, using the gene from clinically relevant strains;
10) *gag*IIIB;

11) tPA-gp120IIIB;
12) gp160 with structural mutations including V3 loop substitutions from clinically relevant strains of HIV; several mutations on several constructs such as variable loop removal, Asn mutations to remove steric carbohydrate obstacles to structural, neutralizing antibody epitopes; and CD4 binding site knockout mutants;
13) gp41 with provision of appropriate leader sequences, as in the tPA signal peptide leader sequence;
14) *gag*: similar to construct from #5 above, using the gene from clinically relevant strains;
15) *rev*: for gp160 and *gag* dicistronics;
16) B7 coding sequences;
17) GM-CSF sequences;
18) Interleukin sequences;
19) Tumor associated antigens;
20) Genes encoding antigens expressed by pathogens other than HIV, such as, but not limited to, influenza virus nucleoprotein, hemagglutinin, matrix, neuraminidase, and other antigenic proteins; herpes simplex virus genes; human papillomavirus genes; tuberculosis antigens; hepatitis A, B, or C virus antigens; and combinations of these and other antigens to form at least dicistronic constructs which may be combined with multiple other polycistronic constructs to provide a cocktail composition capable of raising immune responses against all of the represented pathogens or tumor antigens;

wherein the segments A and B of figure 2 are internal ribosome entry sites or a combination of transcription termination sequences terminating the transcription of the upstream cistron and transcriptional promoter sequences, initiating the transcription of downstream cistron.